

WHAT IS CLAIMED IS:

1 1. A charge-switch nucleotide phosphate (NP) probe, said NP probe
2 comprising:

3 an intact NP probe having a terminal phosphate with a fluorophore moiety
4 attached thereto, said intact NP probe having a first molecular charge associated therewith,
5 whereupon cleavage of said terminal phosphate as a phosphate fluorophore moiety, said
6 phosphate fluorophore moiety carries a second molecular charge, wherein the difference
7 between said first molecular charge and said second molecular charge is at least 0.5.

1 2. The charge-switch NP probe according to claim 1, wherein either said
2 intact NP probe has a positive molecular charge, or wherein upon cleavage of said terminal
3 phosphate fluorophore moiety, said terminal phosphate fluorophore moiety carries a
4 molecular positive charge relative to said intact NP probe.

1 3. The charge-switch NP probe according to claim 1, wherein said
2 charge-switch NP probe is a nucleotide triphosphate (NTP); and wherein said terminal
3 phosphate is a pyrophosphate with a fluorophore moiety attached thereto.

1 4. The charge-switch NP probe according to claim 3, wherein said intact
2 NTP probe has a positive charge.

1 5. The charge-switch NP probe according to claim 3, wherein upon
2 cleavage of said terminal phosphate as a pyrophosphate fluorophore moiety, said
3 pyrophosphate fluorophore moiety carries a positive charge relative to said intact NTP probe.

1 6. The charge-switch NP probe according to claim 3, wherein said NTP
2 probe is a member selected from the group consisting of a deoxynucleotide triphosphate
3 (dNTP), and a nucleotide triphosphate (NTP).

1 7. The charge-switch NP probe according to claim 6, wherein said NTP
2 probe is a deoxynucleotide triphosphate (dNTP).

1 8. The charge-switch NP probe according to claim 7, wherein said
2 deoxynucleotide triphosphate (dNTP) is a member selected from the group consisting of
3 deoxyadenosine triphosphate, deoxycytosine triphosphate, deoxyguanosine triphosphate
4 deoxythymidine triphosphate and deoxyuridine triphosphate.

1 9. The charge-switch NP probe according to claim 6, wherein said
2 nucleotide triphosphate (NTP) is a member selected from the group consisting of adenosine
3 triphosphate, cytosine triphosphate, guanosine triphosphate and uridine triphosphate.

1 10. The charge-switch NP probe according to claim 1, wherein said
2 fluorophore moiety is a member selected from the group consisting of fluorescein, 5-
3 carboxyfluorescein (FAM), rhodamine, 5-(2'-aminoethyl) aminonaphthalene-1-sulfonic acid
4 (EDANS), anthranilamide, coumarin, terbium chelate derivatives, Reactive Red 4, BODIPY
5 dyes and cyanine dyes.

1 11. The charge-switch NP probe according to claim 3, wherein said
2 fluorophore moiety is attached to said terminal phosphate via a linker.

1 12. The charge-switch NP probe according to claim 11, wherein said
2 fluorophore linker is an alkylene group having between about 5 to about 12 carbons.

1 13. The charge-switch NP probe according to claim 11, wherein said linker
2 carries at least one positive charge.

1 14. The charge-switch NP probe according to claim 11, wherein said linker
2 carries at least two positive charges.

1 15. The charge-switch NP probe according to claim 1, wherein at least one
2 of the phosphate moieties of said nucleotide phosphate probe has an ionized oxygen atom
3 with a counter-cation associated therewith.

1 16. The charge-switch NP probe according to claim 15, wherein said
2 counter-cation is a metal ion.

1 17. The charge-switch NP probe according to claim 16, wherein said metal
2 ion is selected from the group consisting of Mg^{++} , Mn^{++} , K^{+} and Na^{+} .

1 18. The charge-switch NP probe according to claim 11, wherein said
2 fluorophore moiety is BODIPY TR.

1 19. The charge-switch NP probe according to claim 1, wherein the
2 difference between said first molecular charge and said second molecular charge is selected

from the group consisting of 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, and 4.0.

20. The charge-switch NP probe according to claim 1, wherein said difference between the first molecular charge and the second molecular charge is calculated in pure water at pH 7.0.

21. The charge-switch NP probe according to claim 1, wherein said charge-switch probe is selected from the group consisting of compound 50, 51, 52, 53, 54, 55, 56, 57, 58, 59 and 60 in Figures 6A-D.

22. A method for separating a labeled nucleotide phosphate having a detectable moiety from a released charged detectable moiety in a sample stream, said method comprising:

a) immobilizing a complex comprising a nucleic acid polymerase or a target nucleic acid onto a solid support in a single molecule configuration;

b) contacting said complex with a sample stream comprising a target nucleic acid when said polymerase is immobilized or a polymerase when said target nucleic acid is immobilized, a primer nucleic acid which complements a region of said target nucleic acid; and a labeled nucleotide phosphate having a detectable moiety, wherein said detectable moiety is released as a charged detectable moiety when said NP is incorporated into said primer nucleic acid; and

c) applying an energy field to said sample stream, thereby separating said labeled NP from said charged detectable moiety.

23. The method according to claim 22, wherein said labeled nucleotide phosphate (NP) is a labeled nucleotide triphosphate (NTP).

24. The method according to claim 23, wherein said labeled nucleotide triphosphate (NTP) having a detectable moiety is a NTP having a γ -phosphate with a detectable moiety attached thereto.

25. The method according to claim 23, wherein said charged detectable moiety when released comprises a pyrophosphate with a fluorophore moiety attached thereto.

1 **26.** The method according to claim **24**, wherein said labeled NTP is
2 incorporated into said nucleic acid primer hybridized to said target nucleic acid using said
3 polymerase, thereby releasing said γ -phosphate with said detectable moiety attached thereto.

1 **27.** The method according to claim **26**, wherein said target nucleic acid
2 comprises a self-complementary region forming said primer.

1 **28.** The method according to claim **22**, wherein the charge of said
2 detectable moiety after release is different than said labeled nucleotide phosphate (NP)
3 having a detectable moiety attached thereto.

1 **29.** The method according to claim **28**, wherein the charge of said
2 detectable moiety is more positive than the unincorporated labeled NP.

1 **30.** The method according to claim **28**, wherein the charge of said
2 detectable moiety attached thereto is opposite in sign compared to the unincorporated
3 fluorescently labeled NP.

1 **31.** The method according to claim **22**, further comprising
2 d) measuring said detectable moiety with a measuring device.

1 **32.** The method according to claim **31**, wherein said measuring device is
2 selected from the group consisting of a charge coupled device (CCD) camera, a photodiode, a
3 video chip, amp meter, voltage meter, and a dye-impregnated polymeric coating on optical
4 fiber sensor.

1 **33.** The method according to claim **32**, wherein said detection is via a
2 CCD camera.

1 **34.** The method according to claim **32**, wherein said detection is via a
2 photodiode.

1 **35.** An analytical method for separating an intact NP probe from a
2 phosphate detectable moiety, said method comprising:
3 a) providing a sample comprising an intact NP probe with a detectable
4 moiety attached thereto, whereupon enzymatic cleavage of said intact NP probe, which

5 produces a phosphate detectable moiety, said phosphate detectable moiety carries a molecular
6 charge which is different than the molecular charge of said intact NP probe; and

7 b) applying an energy field to said sample, thereby separating said
8 phosphate detectable moiety from said intact NP probe.

1 36. The method according to claim 35, wherein said NP probe with a
2 detectable moiety is a labeled nucleotide triphosphate (NTP).

1 37. A method for sequencing a target nucleic acid with a polymerase, said
2 method comprising:

3 a) immobilizing a complex comprising a nucleic acid polymerase or a
4 target nucleic acid onto a solid support in a single molecule configuration;

5 b) contacting said complex with a sample stream comprising a target
6 nucleic acid when said polymerase is immobilized or a polymerase when said target nucleic
7 acid is immobilized, a primer nucleic acid which complements a region of said target nucleic
8 acid of the region to be sequenced; and a labeled nucleotide phosphate having a detectable
9 moiety, wherein said detectable moiety is released as a charged detectable moiety when said
10 NP is incorporated into said primer nucleic acid wherein said solid support is attached to a
11 flowcell having an inlet port and an outlet port;

12 c) applying an energy field to said sample stream; and

13 d) detecting said charged detectable moiety, thereby sequencing said
14 target nucleic acid.

1 ~~38~~ 40. The method according to claim 37, wherein said detectable nucleotide
2 phosphate is a labeled nucleotide triphosphate (NTP) having a γ -phosphate with a detectable
3 moiety attached thereto.

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1 ³⁹~~41~~. The method according to claim 37, wherein said NP is incorporated on
2 said primer strand hybridized to said target nucleic acid using said polymerase and thereby
3 releasing said γ -phosphate with said detectable moiety attached thereto.

1 ⁴⁰~~42~~. The method according to claim 37, wherein said energy field is an
2 electric field.

1 ⁴¹~~43~~. The method according to claim ⁴⁰~~42~~, wherein said electric field is a first
2 electric field applied in the transverse direction and a second electric field applied in the axial
3 direction.

1 ⁴²~~44~~. The method according to claim 37, wherein the charge of said γ -
2 phosphate with said fluorophore moiety attached thereto is greater than the unincorporated
3 fluorescently labeled NTP.

1 ⁴³~~45~~. The method according to claim 37, wherein the charge of said γ -
2 phosphate with said fluorophore moiety attached thereto is less than the unincorporated
3 fluorescently labeled NTP.

1 ⁴⁴~~46~~. The method according to claim 37, wherein the charge of said γ -
2 phosphate with said fluorophore moiety attached thereto is opposite in sign compared to the
3 unincorporated fluorescently labeled NTP.

1 ⁴⁵~~47~~. The method according to claim 37, wherein said detection is via a
2 charge coupled device (CCD) camera.

1 ⁴⁶~~48~~. The method according to claim 37, wherein said detection is via a dye-
2 impregnated polymeric coating on optical fiber sensor.

1 ⁴⁷~~49~~. The method according to claim 37, wherein said detection is via a
2 blockade current.

1 ⁴⁸~~50~~. The method according to claim 37, wherein said detection is via a
2 photodiode.